

What is claimed is:

1. A method of preparing a recombinant adenoviral genome having an insertion nucleic acid located in an E gene region of said genome, said method comprising:

5 (a) providing a first vector comprising an adenoviral genome having an E gene deletion, where said first vector is further characterized by having a second restriction endonuclease site flanked by first and third restriction endonuclease sites, wherein each of said first, second and third restriction endonuclease sites are: (i) different, (ii) do not occur in the corresponding wild type adenoviral genome and (iii) are located in said E  
10 gene deletion region;

(b) providing a second vector comprising an insertion nucleic acid flanked by said first and third restriction endonuclease sites;

(c) contacting each of said first and second vectors with said first and third restriction endonucleases to produce first and second restriction endonuclease cleavage  
15 products, wherein said first cleavage product comprises said adenoviral genome and said second cleavage product comprises said insertion nucleic acid;

(d) combining said first and second cleavage products under ligation conditions sufficient to produce a ligation product composition comprising a ligation product that includes said recombinant adenoviral genome having an insertion nucleic  
20 acid located in an E gene region of said genome;

whereby said recombinant adenoviral genome having an insertion nucleic acid located in an E gene region of said genome is produced.

2. The method according to Claim 1, wherein said method further comprises

25 contacting said ligation product composition with said second restriction endonuclease.

3. The method according to Claim 1, wherein said first and second vectors are plasmids.

4. The method according to Claim 1, wherein said ligation product is a plasmid.
5. The method according to Claim 1, wherein said E gene region is characterized by having a deletion of at least a portion of at least one of an E1, E3 and E4 region.

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6. The method according to Claim 1, wherein said first, second and third restriction endonucleases are selected from the group consisting of I-CeuI, PI-SceI and SwaI.

7. The method according to Claim 1, wherein said second vector is subjected to a purification step prior to said combining step.

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8. The method according to Claim 1, wherein said second vector is not subjected to a purification step prior to said combining step.

9. A method of preparing a recombinant adenoviral genome having an insertion nucleic acid located in an E gene region of said genome, said method comprising:
- (a) providing a first plasmid comprising an adenoviral genome having an E gene deletion region characterized by a deletion of at least one of an E1, E3 and E4 gene, where said first plasmid is further characterized by having a second restriction endonuclease site flanked by first and third restriction endonuclease sites, wherein each of said first, second and third restriction endonuclease sites are: (i) different, (ii) are selected from the group consisting of I-CeuI, PI-SceI and SwaI and (iii) are located in said E gene deletion region;

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- (b) providing a second plasmid comprising an insertion nucleic acid flanked by said first and third restriction endonuclease sites;

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- (c) contacting each of said first and second vectors with said first and third restriction endonucleases to produce first and second cleavage products, wherein said first cleavage product that includes said adenoviral genome and said second cleavage product comprises said insertion nucleic acid;

(d) combining said first and second cleavage products under ligation conditions sufficient to produce a ligation product composition comprising plasmid comprising said recombinant adenoviral genome having an insertion nucleic acid located in an E gene region of said genome; and

5 (e) contacting said ligation product composition with said second restriction endonuclease;

whereby said recombinant adenoviral genome having an insertion nucleic acid located in an E gene region of said genome is produced.

10 10. The method according to Claim 9, wherein said insertion nucleic acid encodes a product.

11. The method according to Claim 9, wherein said method further comprises isolating said second cleavage product prior to said combining step.

15 12. The method according to Claim 9, wherein said adenoviral genome is an AD type 5 genome.

20 13. An adenoviral genome comprising an E gene deletion region and first, second and third restriction endonuclease sites, wherein each of said first, second and third restriction endonuclease sites are: (i) different, (ii) do not occur in the corresponding wild type adenoviral genome and (iii) are located in said E gene deletion region.

25 14. The genome according to Claim 13, wherein said first, second and third restriction endonuclease sites correspond to endonucleases selected from the group consisting of: I-CeuI, PI-SceI and SwaI.

15. The genome according to Claim 13, wherein said E gene region is characterized by having a deletion of at least a portion of at least one of an E1, E3 and E4 region.

16. The genome according to Claim 13, wherein said genome is an AD type 5 genome.

5 17. The genome according to Claim 13, wherein said genome is present on a vector.

18. The genome according to Claim 17, wherein said vector is a plasmid.

19. An adenoviral genome comprising:

- 10 (a) an E gene deletion region;
- (b) first and second restriction endonuclease sites, wherein said first and second restriction endonuclease sites are: (i) different, (ii) do not occur in the corresponding wild type adenoviral genome and (iii) are located in said E gene deletion region; and
- 15 (c) an insertion nucleic acid positioned between said first and second restriction endonuclease sites.

20. The genome according to Claim 19, wherein said first and second restriction endonuclease sites correspond to endonucleases selected from the group consisting of: I-CeuI, PI-SceI and SwaI.

21. The genome according to Claim 19, wherein said E gene region is characterized by having a deletion of at least a portion of at least one of an E1, E3 and E4 region.

25 22. The genome according to Claim 19, wherein said genome is an AD type 5 genome.

23. The genome according to Claim 19, wherein said insertion nucleic acid encodes a product.

24. The genome according to Claim 19, wherein said genome is present on a vector.

25. The genome according to Claim 24, wherein said vector is a plasmid.

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26. A virus comprising a genome according to Claim 13.

27. A virus comprising a genome according to Claim 19.

10 28. A method of producing a recombinant adenovirus having a genome having an insertion nucleic acid located in an E gene region of said genome, said method comprising:

(a) providing a first vector comprising an adenoviral genome having an E gene deletion, where said first vector is further characterized by having a second restriction  
15 endonuclease site flanked by first and third restriction endonuclease sites, wherein each of said first, second and third restriction endonuclease sites are: (i) different, (ii) do not occur in the corresponding wild type adenoviral genome and (iii) are located in said E gene deletion region;

(b) providing a second vector comprising an insertion nucleic acid flanked by  
20 said first and third restriction endonuclease sites;

(c) contacting each of said first and second vectors with said first and third restriction endonucleases to produce first and second cleavage products, wherein said first cleavage product comprises said adenoviral genome and said second cleavage product comprises said insertion nucleic acid;

25 (d) combining said first and second cleavage products under ligation conditions sufficient to produce a ligation product composition comprising a ligation product that includes said recombinant adenoviral genome having an insertion nucleic acid located in an E gene region of said genome;

(e) transforming a cell that is transcomplementing for any inactivated E genes of said E gene region with said ligation product; and

(f) maintaining said transformed cell under conditions sufficient for said virus to be produced;

5 whereby said recombinant adenovirus having a genome having an insertion nucleic acid located in an E gene region of said genome is produced.

29. The method according to Claim 28, wherein said method further comprises contacting said ligation product composition with said second restriction endonuclease  
10 prior to said transforming step.

30. The method according to Claim 28, wherein said first and second vectors are plasmids.

15 31. The method according to Claim 28, wherein said ligation product is a plasmid.

32. The method according to Claim 28, wherein said E gene region is characterized by having a deletion of at least a portion of at least one of an E1, E3 and E4 region.

20 33. The method according to Claim 28, wherein said first, second and third restriction endonucleases are selected from the group consisting of I-CeuI, PI-SceI and SwaI.

34. The method according to Claim 28, wherein said insertion sequence encodes a product.  
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35. The method according to Claim 28, wherein said virus is a type 5 adenovirus.

36. A kit for use in preparing a recombinant adenovirus, said kit comprising:

(a) a first vector comprising an adenoviral genome comprising an E gene deletion region and first, second and third restriction endonuclease sites, wherein each of said first, second and third restriction endonuclease sites are: (i) different, (ii) do not occur in the corresponding wild type adenoviral genome and (iii) are located in said E gene deletion region; and

(b) first, second and third restriction endonucleases that correspond to said first, second and third restriction endonuclease sites.

37. The kit according to Claim 36, wherein said first vector is a plasmid.

38. The kit according to Claim 36, wherein said kit further comprises a shuttle vector comprising a multiple cloning site flanked by said first and second restriction endonuclease sites.

39. The kit according to Claim 38, wherein said shuttle vector is a plasmid.

40. The kit according to Claim 36, wherein said kit further comprises a cell that is transcomplementing for any inactivated E genes of said E gene region with said ligation product